Are *Litoria aurea* eggs more sensitive to ultraviolet-B radiation than eggs of sympatric *L. peronii* or *L. dentata*?

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ABSTRACT

Global declines in amphibian populations in often pristine habitats have led to suggestions that a ubiquitous agent might be involved, such as increased Ultraviolet-B (280–320 nm) radiation due to ozone depletion. The Green and Golden Bell Frog *Litoria aurea*, has suffered marked population declines and is presently listed as an endangered species in New South Wales. The effect of UV-B levels on hatching success in *L. aurea* and two sympatric species which have stable populations (*L. dentata* and *L. peronii*) was tested at two elevations (sea-level and 600 m) and under three UV-B treatments: 1) unfiltered sunlight, 2) sunlight filtered for UV-B, and 3) sunlight filtered for smaller wavelengths. Hatching success was significantly higher under the UV-B-blocking treatment than under the unfiltered treatment in a repeat experiment involving one *L. aurea* spawn ($p = 0.017$), but was not significantly different in the primary experiment involving three *L. aurea* spawns ($p = 0.749$). There were no significant differences between elevations or interactions between treatment and elevation in any species. There was a significant difference in survival between species ($p = 0.001$), with 46% of *L. aurea* surviving *vs* 76% each of *L. dentata* and *L. peronii*. The lack of a coherent trend in hatching success suggests that UV-B irradiance is not affecting survival in the egg to tadpole stage of *L. aurea*.

INTRODUCTION

Amphibian population declines have been documented in Australia, the USA, South America, Canada, and Europe (Barinaga 1990; Czechura and Ingram 1990; Phillips 1990; Blaustein and Wake 1990; Wake 1991; Tyler 1991; Osborne 1994; Pounds and Crump 1994; Trenerry *et al.* 1994). Many of the amphibian population declines have been in montane areas, and at sites not subject to severe habitat disturbance such as National Parks and World Heritage Areas (Fellers and Drost 1994; Richards *et al.* 1993). The declines are species specific; stable and declining species may be members of the same genera, and coexist in the same area (e.g., *Litoria dentata* and *L. aurea* in New South Wales), indicating increased susceptibility on the part of some species due to behavioural, ecological or physiological differences (Wake and Morowitz 1991).

The global nature of the declines led to suggestions that the cause of the declines may be a ubiquitous agent such as increased Ultraviolet-B (UV-B) (280–320 nm) radiation due to decreasing ozone (O_3) levels (Carey 1993; Blaustein *et al.* 1994). As a result of anthropogenic chlorofluorocarbon emissions, global stratospheric O_3 levels have declined in a nearly linear fashion from 1979 to 1991 (Herman and Larko 1994), with decreases of 4–6% per decade at mid-latitudes, and 10–12% per decade at higher latitudes. Lately, the rate of decrease has accelerated (Gleason *et al.* 1993).

UV-B increases are not equivalent globally, but are most notable in montane areas (1.9–5.3% per year with peak pulses of 37% above normal), in the Antarctic (springtime increases of 500% have been recorded), and in the southern hemisphere where short-term increases of UV-B radiation of 12% were recorded after the breakup of the polar vortex sent ozone-poor air over Australia (Lubin and Frederick 1989; Blumthaler and Ambach 1990; Roy *et al.* 1990; Beaglehole and Carter 1992; Lubin *et al.* 1992; Kerr and McElroy 1993; Blumthaler *et al.* 1994a). The latter sites have levels of industrialization which are insufficient to produce compensatory amounts of tropospheric O_3 or atmospheric pollutants (Bruhl and Crutzen 1989; Liu *et al.* 1991). Any increase in UV-B irradiance is of concern as UV-B is strongly absorbed by deoxyribonucleic acids (DNA), damaging DNA by linking adjacent thymine nucleotides (Lloyd 1993).

There is some experimental support for the hypothesis that increases in UV-B levels may be responsible for some amphibian declines. Blaustein *et al.* (1994) conducted an experiment involving two declining and one stable frog species. Eggs from each of the three species were exposed to one of three treatments: 1) unfiltered sunlight, 2) sunlight filtered with a 100% UV-B blocking filter, and 3) a control filter which transmitted 80% UV-B. The hatching success of the stable species did not improve when UV-B irradiance was blocked, however, hatching success almost doubled in the two declining species when the UV-B blocking filter was in place compared to those under unfiltered sunlight. A proportional improvement in hatching success in the declining species was noted when the control filter which blocked 20% UV-B was in place. Damage to DNA is a normal

Table 1. Location, elevation and characteristics of the field sites used to study hatching success in *Litoria aurea*, *L. dentata* and *L. peronii* under three UV-B treatments in December 1994 to February 1995.

Site name (abbreviated)	Latitude and Longitude	Elevation (m)	Water temperature (°C) (n = 10)*	pH (n = 3)*
Uni. of Wollongong Duck pond 1 (DP1)	150°52′E, 34°24′S	28	23.5 ± 0.6	8.2 ± 1.0
Uni. of Wollongong Duck pond 2 (DP2)	150°52′E, 34°24′S	30	23.1 ± 0.9	8.3 ± 0.4
Botanical Gardens, Wollongong (BG)	150°52′E, 34°24′S	18	24.6 ± 0.5	8.1 ± 0.9
BHP Refractory, Thirroul (T)	150°55′E, 34°19′S	3	24.7± 0.8	7.8 ± 0.8
Robertson, Dam 1 (R1)	150°36′E, 34°37′S	560	22.3 ± 0.9	6.2 ± 0.4
Robertson, Dam 2 (R2)	150°36′E, 34°37′S	572	23.0 ± 0.6	7.0 ± 0.3
Robertson, Dam 3 (R3)	150°36′E, 34°37′S	583	22.8 ± 0.6	7.0 ± 0.3
Robertson, Dam 4 (R4)	150°36′E, 34°37′S	590	21.9 ± 0.5	7.1 ± 0.3

*mean ± SE

consequence of exposure to UV-B radiation in all living organisms, however, the extent of the damage is limited by photoreactivation repair enzymes such as photolyase (Blaustein *et al.* 1994). An assay of photolyase showed that levels were three to six times lower in the frog species which showed signs of populational declines.

The Green and Golden Bell Frog *Litoria aurea* has suffered both marked range reduction and population declines over the past 20 years (White 1995). It has disappeared from elevated sites in the Northern Tablelands and Southern Highlands of New South Wales, and contracted to a small number of populations found mostly around large population centres such as Sydney, Wollongong, and Newcastle (Osborne 1990; Daly 1995; White 1995). The animal is now listed as an Endangered Species in New South Wales (White 1995). By contrast, the populations in Victoria appear to be stable (Gillespie 1995).

Given that UV-B irradiance increases with elevation (Blumthaler *et al.* 1994), that UV-B levels decrease in industrialized, urban environments (Bruhl and Crutzen 1989; Liu *et al.* 1991), that UV-B levels have been shown to have increased in southern Australia (Atkinson *et al.* 1989; Roy *et al.* 1990), and that *L. aurea* — which lay their spawn in shallow water often highly exposed to sunlight (pers. obs.) — have disappeared from montane areas (Osborne 1990) whilst remaining in urban environments (White and Pyke 1996), it was decided to test the hypothesis that UV-B was adversely affecting *L. aurea* populations.

We conducted an experiment similar to that of Blaustein *et al.* (1994), but included sites at approximately 600 m altitude in addition to low altitude sites to see if there was an elevational gradient in the effects of UV-B. Hatching success under three UV-B treatments was monitored, comparing *L. aurea* with two species whose populations appear to be stable: *L. dentata* and *L. peronii*. If the findings of Blaustein *et al.* (1994) were applicable to the Australian amphibian population, we would expect hatching success to improve when egg exposure to UV-B levels was reduced either by decreasing the altitude of the site of exposure or by filtering the UV-B wavelengths. Accordingly, the null hypotheses tested were: 1) hatching success would be equivalent at lowland and elevated sites within each species, and 2) reduced exposure to UV-B radiation would not increase hatching success.

METHODS

Sites

Field experiments were conducted at eight sites in the Illawarra and Southern Highlands regions of New South Wales, Australia. All sites were located within the known range of *L. aurea* (Cogger 1992). The four coastal sites were located between Thirroul and Wollongong and were situated at elevations ranging from 3–30 m above sea-level (Table 1). The sites in the Southern Highlands were located 10 km south-east of Robertson at 560–590 m elevation. Depending on the aerosol content of the atmosphere and the ground albedo (reflectivity), a difference in altitude of 500 m should result in an increase in UV-B irradiance of 4–14%, due to the decrease in the solar path length through the atmosphere (Blumthaler *et al.* 1994).

All sites were artificial bodies of water: four farm dams, one siltation dam at an industrial site, and three ornamental ponds in the grounds of public institutions (Table 1). Six of the sites contained *Typha domingensis* as the main aquatic vegetation. All sites received unrestricted sunlight between 1100 h and 1600 h.

Experimental Design

At each site a raft was constructed of aluminium, with plastic drainage pipes serving as a pontoon.

Each raft contained nine perspex boxes (40 cm w × 40 cm l × 7 cm h) with 1 mm^2 fibreglass mesh bases. A muslin insert was laid on top of the mesh to prevent egg/tadpole losses. At each site there were three treatments for each of three species. The treatments were 1) unfiltered sunlight, 2) sunlight filtered to remove UV-B, and 3) sunlight filtered to allow 80% transmission of UV-B. The UV-B blocking filter was made of Mylar and the control filter was polyethylene. The transmissivity of the filters was evaluated before and after the experiments using a Carey 17 spectrophotometer. The Mylar blocked 100% of UV-B between 280 and 315 nm. Ten per cent of UV-B irradiance was transmitted at 320 nm. The polyethylene filter allowed 80% transmission of UV-B whilst new, however, after three months in the field it allowed about 50% transmission of wavelengths between 280 and 320 nm.

One-hundred-and-fifty freshly laid frog eggs from each of 3–10 spawns from different amplecting pairs were placed in each box (Table 2). The number of eggs from a given spawn were equivalent in each box. The boxes were placed on the rafts to a depth of 2 cm. Tadpoles were counted and removed daily until all eggs either hatched or died. The experiments took place between January and February 1995. Water temperature, turbidity, and pH were measured at all sites during the course of the experiment (Table 1). The water at all sites contained <30 mg/L of dissolved solids.

Four small pilot experiments were carried out in November–December 1994, to deal with field problems. The results of these experiments are not reported here. The *L. aurea* experiment was carried out twice. The second experiment used identical methods to the first, except it was carried out with only one spawn (Table 2). Observations of animal sizes were recorded in order to contribute to the knowledge of the species.

Table 2. The dates of UV-B experiments and the number of spawns per experiment for *Litoria aurea, L. dentata,* and *L. peronii.*

Species	Date experiment commenced	No. Spawns per experiment
Litoria aurea#1	6/1/95	3
Litoria dentata	21/1/95	10
Litoria peronii	26/1/95	5
Litoria aurea#2	13/2/95	1

#experiment no.

Collection of Spawn

Thirteen amplecting pairs of *L. aurea* were collected on four occasions between 25/12/94 and 12/2/95 at a site in Port Kembla. The duration of amplexus ranged from eight hours to three days, although five pairs of *L. aurea* which had not spawned after three days were released still in amplexus. Each amplecting pair was housed in individual polystyrene boxes containing water and some aquatic weed or grass (Daly 1996). The boxes were tilted at an angle to provide a range of water depths. Spawns were collected from the boxes, counted and transferred to the experimental boxes. Spawns were kept in pond water in plastic containers during transfer.

Eleven pairs of *L. dentata* were collected at Helensburgh on the 21/1/95. Eight amplecting pairs of *L. peronii* were collected between 21/11/94 and the 2/2/95 (four provided by G. Daly). The duration of amplexus for both species was less than eight hours.

Statistical Analysis

An arcsine of the square root transformation was carried out on the data to yield a normal distribution (Zar 1984). A two factor ANOVA was used to test the effect of elevation and treatment on each species. A Bonferroni test and Tukey's multiple comparison test were carried out on the data to determine which means were significantly different from each other. Percentage survival for the three species was compared via a single factor ANOVA, as differences in breeding times and possible differences in UV-B exposure made use of a three factor ANOVA inappropriate. All variance statistics presented are standard errors, and sample size is denoted as n.

RESULTS

Observations

The snout-vent lengths of *L. aurea* males were 61 to 75 mm (mean 67.6 ± 1.5 mm; n = 10) and that of females 66 to 84 mm (mean 75.7 ± 1.9 mm; n = 11). *Litoria dentata* males were 39 to 43 mm in length (mean 40.8 ± 0.4 mm; n = 10) and females were 42 to 46 mm (mean 44.0 ± 0.4; n = 10). *Litoria peronii* males were 46 to 60 mm in length (mean 53.7 ± 1.3 mm; n = 11) while females were 56–71 mm long (mean 63.1 ± 1.9; n = 8).

Spawn Sizes

Litoria aurea spawns ranged in size from 4 124 to 6 178 eggs (mean 5 109 ± 328; n = 5), while those of *L. dentata* consisted of 238 to 1881 eggs (mean 755.8 ± 196.7; n = 8), and those of *L. peronii* contained 490 to 1 869 eggs (mean 1 078.8 ± 177.2; n = 8).

Hatching Times

Of those eggs which hatched, 86% of *L. aurea* eggs hatched in two days, while 11% took three days and 3% took four days to hatch, when the mean water temperature was 24.0 ± 0.5°C (n = 26). The percentage of *L. dentata* eggs

Table 3. Two factor ANOVA for the effects of treatment and elevation on hatching success in three frog species: *Litoria aurea, L. dentata* and *L. peronii*. *significant at 0.05.

Species	Source	df	F-test	P value
L. aurea#1	Elevation	1	0.84	0.40
	Treatment	2	1.30	0.31
	Interaction	2	1.41	0.28
L. dentata	Elevation	1	3.37	0.12
	Treatment	2	0.30	0.75
	Interaction	2	0.26	0.78
L. peronii	Elevation	1	0.30	0.61
	Treatment	2	1.43	0.28
	Interaction	2	0.33	0.72
L. aurea#2	Elevation	1	0.66	0.45
	Treatment	2	5.89	0.02*
	Interaction	2	1.74	0.22

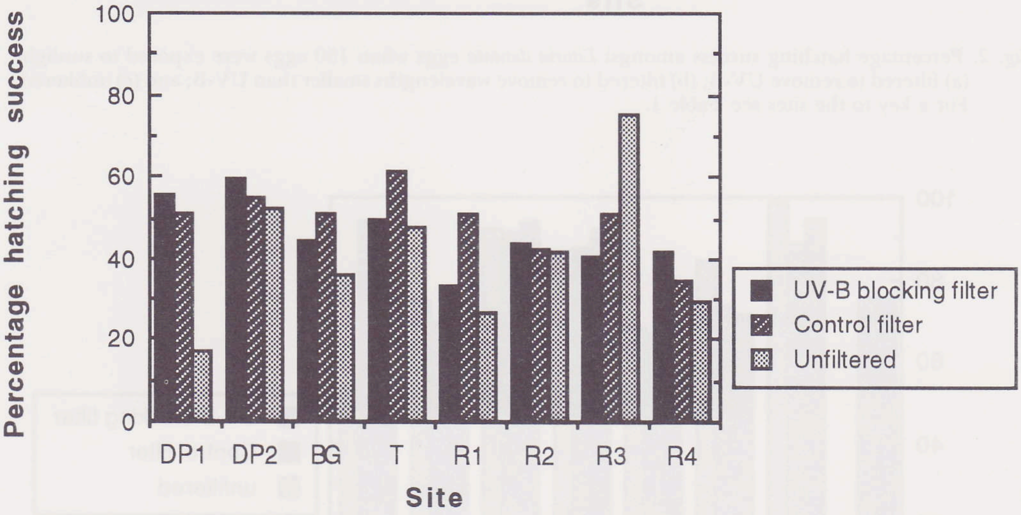

Fig. 1. Percentage hatching success amongst *Litoria aurea* eggs when 150 eggs were exposed to sunlight (a) filtered to remove UV-B; (b) filtered to remove wavelengths smaller than UV-B; and (c) unfiltered. For a key to the sites see Table 1.

which hatched were 54%, 19%, 27%, 0.5% and 0.1% on days two, three, four, five and nine respectively, when the mean water temperature was 20.5 ± 0.4°C (n = 12). Sixty-two per cent of *L. peronii* eggs hatched in three days, 38% took four days and 0.06% took eight days to hatch, when the mean water temperature was 23.4 ± 0.4°C (n = 22).

Treatment Effects

There were no significant differences in hatching success between treatments for any of the three species tested in the first experiment (Table 3). Amongst *L. aurea*, hatching success was greatest under the UV-B-blocking filter at 50% of sites, under the control filter at 38% of sites and under unfiltered sunlight at 13% of sites (Fig. 1). The corresponding values for *L. dentata* were 25%, 38% and 25% respectively, with one site showing equal hatching success between the unfiltered treatment and the treatment with the control filter (Fig. 2). Amongst *L. peronii*, the corresponding values were 38%, 13% and 38% respectively, with one site showing equal hatching success between the UV-B blocked treatment and the control filter (Fig. 3).

When all sites were combined, the greatest number of *L. aurea* tadpoles hatched under the control filters, 1.1 times that for the UV-B-blocking filters and 1.2 times that for the unfiltered treatment. Total hatching success was marginally better under the UV-B-blocking filters amongst *L. dentata* and *L. peronii.*

An additional experiment carried out in February 1995, although using only one *L. aurea* spawn, showed similar results to the January experiment which used eggs from three spawns. Hatching success was still greatest under the UV-B-blocking filter at 50% of sites, but the sites at which this occurred differed from those in the previous experiment. Hatching success was significantly higher under the UV-B-blocking filters than under the unfiltered treatment (p = 0.017).

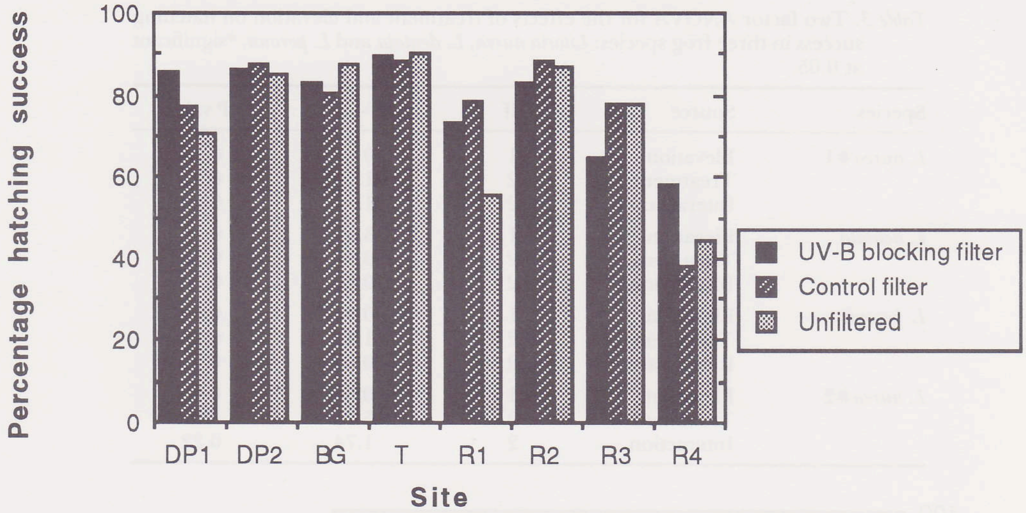

Fig. 2. Percentage hatching success amongst *Litoria dentata* eggs whan 150 eggs were exposed to sunlight (a) filtered to remove UV-B; (b) filtered to remove wavelengths smaller than UV-B; and (c) unfiltered. For a key to the sites see Table 1.

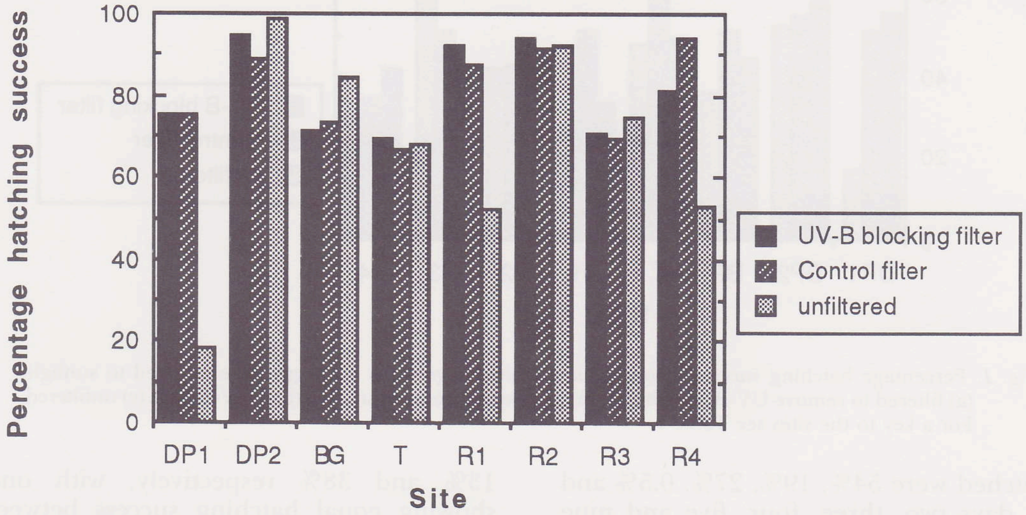

Fig. 3. Percentage hatching success amongst *Litoria peronii* eggs when 150 eggs were exposed to sunlight (a) filtered to remove UV-B; (b) filtered to remove wavelengths smaller than UV-B; and (c) unfiltered. For a key to the sites see Table 1.

Elevation Effects

There were no significant differences in hatching success between the elevated and coastal sites for any of the three species ($p > 0.1$) when all treatments were combined (Table 3). Forty-eight per cent of *L. aurea* eggs survived to the hatchling stage at the coastal sites, *vs* 43% at the elevated sites, but under the unfiltered treatment, survival to hatchling stage was 1.1 times higher at the elevated sites than at the coastal sites (Fig. 4). Survival to hatchling stage was 83% *vs* 69% for the coastal and elevated sites amongst *L. dentata*, and 74% *vs* 79% for *L. peronii*. There were no significant interactions between elevation and treatment for any of the three species (Table 2).

In the second *L. aurea* experiment, hatching success was 37% at coastal sites *vs* 42% at elevated sites. The difference was not significant (Table 3).

Species Effects

Overall survival to hatchling stage differed significantly between species ($p = 0.0001$). Only 46% of *L. aurea* eggs survived to hatchling stage as opposed to 75.9% and 76.4% for *L. dentata* and *L. peronii* respectively. This represents 1.7 times as many hatchlings for both *L. dentata* and *L. peronii* compared to *L. aurea* (Fig. 5). Total survival amongst *L. aurea* in the second experiment was only 40%.

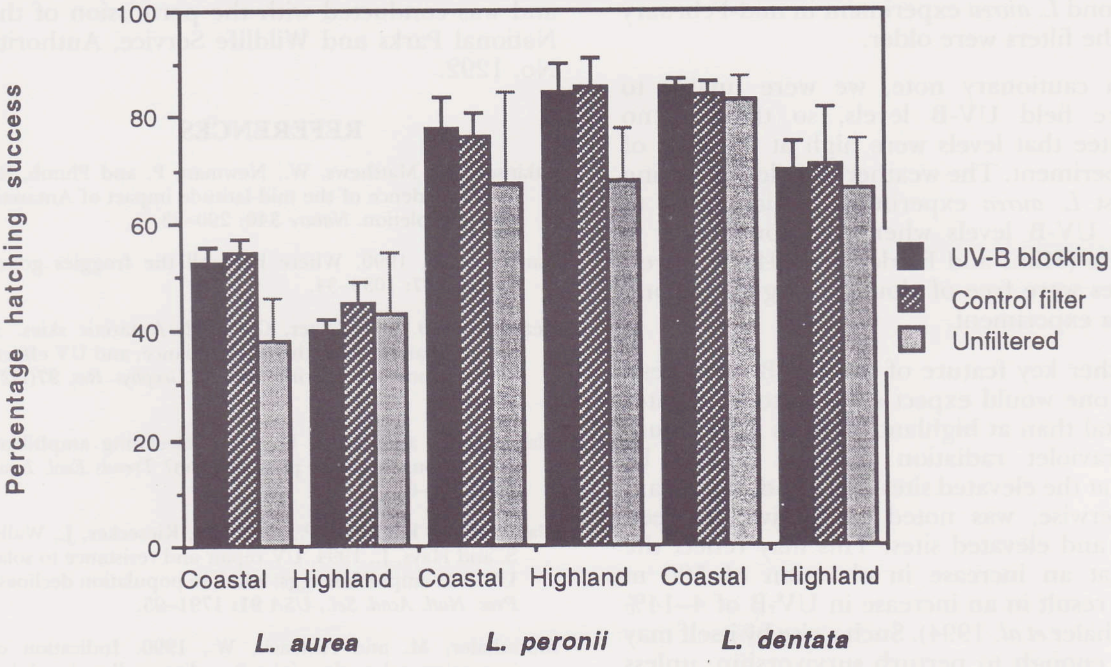

Fig. 4. Percentage hatching success at coastal (3–30 m) and elevated (560–590) sites for *Litoria aurea L. dentata* and *L. peronii.*

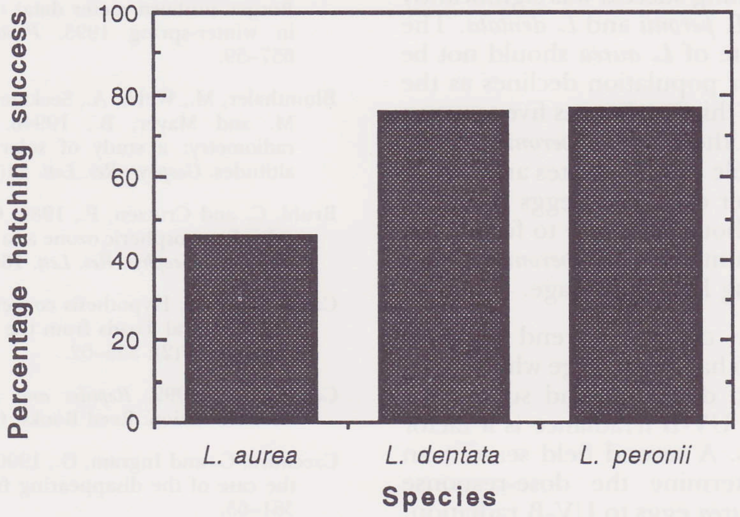

Fig. 5. A comparison of total percentage hatching success between *Litoria aurea, L. dentata* and *L. peroni* when sites and treatments were combined.

DISCUSSION

If the UV-B hypothesis is to be supported, one would expect survival to the hatchling stage to be greatest for *L. aurea* under the UV-B-blocking filter, and lowest under unfiltered sunlight, with the control filter ranging somewhere in between. This was not the case. On a site-by-site basis, hatching success for *L. aurea* was greatest under the UV-B-blocking filter at only 50% of sites, while the total number of hatchlings was greatest under the control filters when sites were combined during the first experiment.

Predation and fouling of unfiltered treatments by water birds may account for the significantly reduced survival under the unfiltered treatments seen in the second *L. aurea* experiment, however the presence of the control filters ensured that greater survival under the experimental filter would not be misread as a UV-B effect rather than an effect of simply having a cover over the treatment.

Transmission of UV-B was reduced from 80% to 50% in the control filters over the period of the experiment, but one would still expect to see a difference in survivorship between the UV-B-blocking and control filters when 50% UV-B was being transmitted. Also, the first *L. aurea* experiment was carried out with new filters in early January, and the results were similar to the second *L. aurea* experiment in mid-February when the filters were older.

As a cautionary note, we were unable to measure field UV-B levels, so there is no guarantee that levels were high at the time of the experiment. The weather was cloudy during the first *L. aurea* experiment, which tends to reduce UV-B levels when the cloud cover is complete (Mims and Frederick 1994), however, the skies were free of cloud during the second *L. aurea* experiment.

Another key feature of the UV-B hypothesis is that one would expect survival to be greater at coastal than at highland sites, as the amount of ultraviolet radiation received should be higher at the elevated sites. No trend, significant or otherwise, was noted in survival between coastal and elevated sites. This may reflect the fact that an increase in elevation of 500 m should result in an increase in UV-B of 4–14% (Blumthaler *et al.* 1994). Such a rise by itself may not be enough to perturb survivorship, unless UV-B levels were already near the species limit of tolerance.

Litoria aurea hatching success was significantly lower than that of *L. peronii* and *L. dentata*. The higher mortality rate of *L. aurea* should not be seen as a reason for population declines as the mean spawn size of this species was five to seven times higher than those of *L. peronii* and *L. dentata*. If comparable hatching rates are seen in the wild, the number of *L. aurea* eggs surviving to hatchling stage should be three to four times higher than the number of *L. peronii* and *L. dentata* eggs reaching hatchling stage.

The lack of a coherent trend towards increased survival to hatchling stage when UV-B levels were reduced does not lend support to the suggestion that UV-B irradiance is a factor in *L. aurea* declines. A second field season; an experiment to determine the dose-response relationship of *L. aurea* eggs to UV-B radiation; and an assay of photolyase levels may confirm these findings.

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